BD1 CIP FWC III

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Sherie L. Morrison, et al.
Serial No.	:	07/893,610
Filed	:	June 3, 1992
For	:	RECEPTORS BY DNA SPLICING AND EXPRESSION
Art Unit	:	1806
Examiner	:	T. Nisbet

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

DECLARATION OF SHERIE L. MORRISON
PURSUANT TO 37 C.F.R. § 1.132

I, SHERIE L. MORRISON, declare that:

1. I am a co-inventor in the above-identified patent application.

2. I make this declaration to clarify the record in response to the examiner's invitation in paragraph 17 of Paper No. 26 and to respectfully traverse the examiner's grounds for rejection under 35 U.S.C. § 103. In this declaration, I set forth and explain laboratory data that establish the unexpectedly high yield of functionally assembled antibody that results from practicing the claimed invention. I further explain why the unexpectedly high

yield of functionally assembled antibody obtained from exogenous co-transformation of the J558L cell line is predictive of similarly high yields at least from other mammalian cell lines.

3. Prior to filing the first parent application in this case, I performed an assay for specific binding upon the cellular expression product from mammalian cells co-transfected with DNA coding for the heavy and light chains of a functional antibody. I labelled the secreted protein with ^{14}C and ran it through a column containing phosphocholine, the antigen for the subject antibody. I then eluted the expression product with soluble antigens.

4. As shown on the copy of the page from my laboratory notebook attached hereto as Exhibit A, the eluate was separated into five pools, each of which was precipitated with one of the following: IgGSorb (immobilized Staphylococcus Protein A which binds human IgG heavy chains), IgGSorb coated with rat monoclonal antibody TC-102.1.2 (designated 120), T156.1.1 (designated 156), or T139.2 (designated 139), and IgGSorb coated with rabbit anti-mouse F_c followed by monoclonal mouse anti-human kappa (designated R4). The rat anti-idiotypic antibodies were culture supernatants. As described in Molecular Immunology, vol. 21, p. 961 (1984), T156.1.1 is an antibody to an

idiotope comprised of the heavy chain alone and TC102.1.2 and T139.2 are antibodies which require both the specific H and L chain to form the idiotope.

5. 21,000 counts were specifically precipitated from the pooled material that had bound to the antigen column, i.e. functional antibody. IgGSorb (Staph A) precipitated 45,000 counts from the flow-through protein that had not bound to the antigen column, i.e. something other than functional antibody. IgGSorb was used because the requirements for its recognition are the least stringent of the above antibody-binding agents and it would be expected to give a maximal estimate of the amount of immunoglobulin-related material not binding to the column. This assay showed an unexpectedly high 32% yield of functionally assembled antibody (21,000 ÷ (21,000 + 45,000)) when compared with the 0.76% yield of "recombined antibody" cited in the Cabilly 4,816,567 patent.

6. Analysis by SDS-PAGE of proteins precipitated after binding to the affinity column verified that the proteins were fully assembled antibody protein. See Morrison et al., Proc. Nat'l. Acad. Sci., USA, vol. 81, p. 6851-6855, fig. 3.

7. The assay that generated the 32% yield was not run under conditions designed to maximize yield. In

fact, that yield was so unexpectedly high that I did not feel a need to take additional steps to maximize yield. Had I intended to maximize yield, I would have passed the flow-through material through the column several times, instead of running the material rapidly through the column only once.

8. It is my opinion that the 32% yield is an underestimate of the amount of correctly assembled antibody because the anti-phosphocholine antibody is inherently a low affinity molecule and under the conditions used in this particular assay I would not expect to observe binding by all of the antibodies that are capable of binding under more suitable conditions. Also, I was not carefully controlling the presence of phosphate in the material, and phosphate interferes with antigen binding.

9. Even if the 32% yield is an underestimate of the proportion of functionally-assembled antibody produced by the transfected cells, the statistical significance of the increased yield when compared with the 0.76% yield cited in the Cabilly patent is certain. Our yield is not just 50 percent or 100 percent greater, but an unexpected 42 times greater than Cabilly's yield. In my opinion it is not possible that this increase could be within the range of experimental error.

10. This increase in yield of functional antibodies is of great practical significance. This higher yield means that a useful amount of a desired functional antibody can be produced allowing easier study of antibody structure and function and facilitating the development of antibody molecules for immunodiagnosis and immunotherapy.

11. The work that I performed with the J558L cell line demonstrates that a mammalian cell line could express, assemble and secrete as functional antibody the product of exogenous heavy and light chain genes when acted upon in accordance with this invention.

12. Because there was no reason to believe that J558L differed from other mammalian cells with respect to its ability to express its endogenous genes (in fact it did produce and secrete a lambda light chain) and because mammalian cells were known to express, assemble and secrete large quantities of antibody protein encoded by endogenous genes, see e.g., Scharff et. al., Molecular Approaches to Immunology, pp. 109-29 (1975); Scharff, Series 69, The Harvey Lectures, pp. 125-42 (1975) (copies attached hereto as Exhibits B and C, respectively), we expected that the expression of exogenous heavy and light chain genes achieved with J558L genes would be predictive of similar expression of exogenous genes with other mammalian cells.

13. In fact, in subsequent experiments by us and many others, mammalian cell lines were found to be capable of efficiently producing fully-assembled functional antibodies when co-transfected with exogenous antibody chain genes. Copies of representative papers evidencing this result are attached hereto as Exhibit D.

14. In the fall of 1984, I met Michael A. Boss at a conference in Florence, Italy where I presented a lecture in which I described the work upon which this application is based. He congratulated me on successfully producing functional antibodies, saying that my group had chosen to work with eukaryotes while he had chosen bacteria and that we had succeeded where he had not.

15. I further declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further that all these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code and that

such willful, false statements may jeopardize the validity of the application or any patent issuing thereon.

By Sherie L. Morrison
Sherie L. Morrison

Dated: August 18, 1993

Exhibit F

MATERIAL SENT FROM L. A. HERZENBERG LABORATORY

Sent to: Sherrie Morrison
Columbia, New York

Material Sent No. 178

Date Sent: 11/23/

Sent By: Tim S

NOTE TO RECEIVER: Please complete the information below and return to:

Ms. Sandy Scaling
c/o Dr. L. A. Herzenberg
Department of Genetics, S-337
Stanford University School of Medicine
Stanford, CA, 94305 USA

Date Received: _____ Material Sent No. _____

Condition of Material When Received: _____

Comments:

MATERIAL SENT		QUANTITY	FORM
~~Cell Line~~	purified DNA .1mls pSV2ΔH1S107 VH-K-7 840 ug/mL 6/26/83 TG		Culture ___ Tumor ___
~~Hybridoma~~ ~~Serum~~	.25mls pSV184 DNSVH-M 615 ug/mL #3 JD		Frozen ___ Serum ___
~~Animal~~ ~~(Strain)~~	Bugs pSV2ΔHS107HuK (from TG ON.) pSV184 DNSVH-M 11/22/83 JL pSV2S107VHHuG-2 (Jacki J.)		Live Animal ___ Other ___
Other			